

Ethanol Influences on Native T-Type Calcium Current in Thalamic Sleep Circuitry

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ABSTRACT

Ethanol is known to disrupt normal sleep rhythms; however, the cellular basis for this influence is unknown. This study uses an *in vitro* slice preparation coupled with electrophysiological recordings to probe neuronal responses to acute ethanol exposure. Recordings were conducted in ferret and rat thalamic slices, since thalamic circuitry is an integral component of sleep/wake cycles and sleep spindles. A key mediator of spindle wave activity is the low-threshold calcium current (T-type current). The T-type current underlies burst responses in the lateral geniculate and thalamic reticular nuclei that are important in spindle propagation. Whole cell patch recordings in thalamic brain slices revealed that ethanol has a differential, dose-dependent effect on the native T-type current in thalamic

relay cells. Low concentrations of ethanol (2.5, 5, and 10 mM) enhance T-type current ($n = 35$), whereas higher concentrations of ethanol (20 and 50 mM) decrease T-type current ($n = 27$). To address whether this dose-dependent effect was due to variation between cells, in a subset we verified the differential effect within the same cell ($n = 7$). In an effort to examine whether the biphasic effects on the current were due to the order of ethanol exposures, we varied the order of high and low ethanol concentrations within the same cell. The ability of ethanol to perturb calcium currents in thalamic relay cells may provide a mechanistic framework for the well documented disruptions in sleep/wake behavior in subjects with ethanol exposure.

The sedative effect of drinking alcohol is well known (Allen et al., 1971; Gillin et al., 1990; Landolt and Gillin, 2001), and for some insomniacs this effect is the pathway to bedtime alcohol consumption and eventual abuse (Roehrs et al., 1999; Brower et al., 2001). Sleep disturbances are reportedly common in alcoholic patients, often resulting in a number of serious health consequences. Perhaps most compelling is that the perturbations in sleep patterns are predictive of relapse in recovering alcoholics (Gillin et al., 1994; Clark et al., 1998; Drummond et al., 1998). Ethanol is known to influence the clinical course of psychiatric and sleep disorders by a number of mechanisms, which have as a common basis perturbations in the balance of neurotransmitter systems or in the function of voltage-gated channels (Castaneda et al., 1998; Nutt, 1999).

The thalamus is the primary relay of sensory information

from the periphery to the cerebral cortex, and in this role it is vital to conscious perception. The thalamus also has a role in sleep, where it is integral to sleep/wake rhythms and the spindle waves that occur during Stage II sleep (Steriade et al., 1985; Steriade and Llinás, 1988). This specific form of sleep is enhanced in response to acute alcohol administration and reduced in alcoholics (Stone, 1980; Williams and Rundle, 1981; Vitiello, 1997; Landolt and Gillin, 2001). Despite this influence of ethanol, little is known regarding the cellular mechanisms underlying any effect of ethanol on sleep. Slices of the ferret thalamus are optimal for exploring such mechanisms, because they possess the necessary circuitry and voltage-dependent properties for the generation of spindle waves *in vitro* (Steriade et al., 1985; Bal et al., 1995a,b; Kim et al., 1995).

An important mediator of spindle rhythms is a low-threshold calcium current (T-type current) that is found in the thalamus. This channel is highly conserved in mammals, and three T-type channel transcripts have been cloned in mice, rats, and humans (for review, see Perez-Reyes, 2002). One of these transcripts, $\alpha.1.G$, is highly enriched in thalamic relay nuclei, the pyramidal cell layer of hippocampus, and in the Purkinje, inferior olive, and posterior granule cell layers of

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ABBREVIATIONS: LTS, low threshold spike; EEG, electroencephalogram; LGN, lateral geniculate nucleus; REM, rapid eye movement; EtOH, ethanol; ACSF, artificial cerebral spinal fluid.

the cerebellum (Talley et al., 1999). In the thalamus, this current underlies the generation of burst responses in individual cells within the spindle-generating circuitry (Jahnsen and Llinás, 1984a,b; Kim et al., 1995; Bal et al., 1995a,b). The T-type current is a transient inward current that is activated by depolarization from a hyperpolarized resting membrane potential (typically -65 mV and more hyperpolarized). Native T-type current shows a property of deinactivation that is both voltage- and time-dependent, imbuing the current with an intrinsic refractory period. This refractory period limits the rate at which successive bursts may occur, and this in turn contributes to the frequency of spindle oscillations recorded as electroencephalographic (EEG) responses at the cortical surface (McCormick and Feuser 1990; Bal et al., 1995a,b; Kim et al., 1995). Thus, understanding the response of T-type current in the thalamus is integral to understanding the fate of spindle oscillations in response to ethanol.

Using whole cell patch recordings in slices of the thalamus, we tested the hypothesis that T-type calcium currents are suppressed by acute administration of intoxicating concentrations of ethanol. Although we confirmed this hypothesis, we also found that the T-type calcium current is exquisitely sensitive to low millimolar concentrations of ethanol, but these lower concentrations increase the amplitude of the current. Our findings suggest important functional consequences of low versus high alcohol consumption on normal sleep patterns.

Materials and Methods

In Vitro Slice Preparation. Ferrets or rats were deeply anesthetized with halothane followed by decapitation and extraction of a block of tissue containing the LGN, in accordance with Wake Forest Animal Care and Use Committee guidelines. The block was immediately immersed in ice-cold buffer (124 mM NaCl, 26 mM NaHCO₃, 10 mM dextrose, 5 mM KCl, 2 mM CaCl₂, 1.25 mM NaH₂PO₄, and 1 mM MgSO₄, pH 7.4). Slices (200- or 400- μ m thick) were cut from the block with a vibratome while the block was immersed in cold buffer. For "blind" recording, 400- μ m slices were placed on a nylon mesh in a recording chamber at an interface of warmed (34°C), oxygenated (95% O₂, 5% CO₂) air and buffer. For visualized recordings, the 200- μ m slices were placed in a chamber on the stage of a Zeiss Axioskop (Thornwood, NY) that was temperature controlled to 34°C.

Recordings. Micropipettes were pulled to a fine tip (30–60 M Ω) and were filled with 4 M potassium acetate. Patch pipettes (6–10 M Ω) were filled with an internal recording solution composed of 128 mM *N*-methyl-D-glucamine, 20 mM tetraethylammonium-Cl, 10 mM Hepes-Na, 2 mM MgCl₂, and 2 mM Na₂-ATP. The internal sodium channel blocker QX314 was added to the patch pipette internal solution to allow us to isolate the low-threshold spike from contaminating Na⁺ spikes in voltage-clamp recordings. Neural activity was recorded either with an Axoclamp 2B (current clamp) or an Axopatch 200A (voltage clamp; Axon Instruments Inc., Foster City, CA). A charge-coupled device camera was used to image the surface of the slice through a 40 \times water immersion objective. The patch pipette tip was pressurized, advanced under the single electrode voltage-clamp mode of the amplifier until the electrode dimpled the cell membrane, and then whole cell access was obtained by rupturing the seal. Recordings made with high impedance electrodes were included if the recording duration exceeded 10 min with a resting membrane potential of -50 mV or less and overshooting action potentials. Pharmacological delivery was performed with bath substitution.

T-Type Ca²⁺ Isolation and Quantification. The low-threshold calcium spike can be recorded with current clamp recordings, and the underlying T-type Ca²⁺ current can be recorded with voltage-

clamp recordings. Low-threshold spikes could be evoked in current clamp mode by depolarizing current pulses executed from a membrane potential more hyperpolarized than ~ -70 mV. In voltage-clamp recordings, a series of voltage step commands between -100 and -40 mV allowed us to characterize the activation and inactivation curves for T-type current (Coulter et al., 1989). All pharmacologic agents are bath-applied by total bath substitution. During the experiment, we switch between multiple perfusion reservoirs containing exactly the same oxygenated ACSF used for the baseline recordings in addition to the concentration of pharmacologic agent listed below to deliver a precise concentration of drug to the tissue section. At the end of drug delivery we "wash" the section in the control ACSF described above. It takes between 1 and 1.5 min for the solution to reach the tissue slice after switching between ACSF reservoirs. Ethanol was bath-applied at concentrations corresponding to physiologically relevant blood alcohol concentrations, 2.5–50 mM (Weiner et al., 1994). At an estimated flow rate of 2 ml per minute, it takes approximately 15 min to achieve washout of ethanol effects using the interface chamber and approximately 10 min at the visualized patch rig. Activation and inactivation measures were taken before, during delivery, and after washout of ethanol. Voltage-clamp control was determined in this and our other studies by smooth, voltage-dependent current activation, the absence of delay in the onset of the current in response to voltage commands, and onset and offset kinetics that were voltage-dependent and which were not affected by the amplitude of the currents. We routinely monitored and recorded the resulting output command voltages recorded at the electrode and examined these for signs of escape from voltage clamp.

Analysis. We plotted activation/inactivation curves resulting from the voltage command protocols to the Boltzmann equation: $G/G_{\max} = 1/(1 + \exp[(V_{1/2} - V)/k])$ to derive, by least-squares fits, the half-maximal voltage ($V_{1/2}$) and slope factor (k) values for comparisons. A Wilcoxon sign rank test and a Student's *t* test were used to assess the significance of population effects.

Results

Whole cell patch-clamp intracellular recordings were obtained from 91 LGN thalamocortical cells from thalamic slices maintained *in vitro*. Seventy-one cells were from adult ferrets, and 20 cells were from adult Sprague-Dawley rats. No differences were observed between the ferret and rat data, and these were pooled for population analysis. All of the figures illustrate data from ferret LGN cells.

Rebound Bursts and Ethanol. The "burst firing" mode of thalamocortical cells during spindle wave sleep is mediated by T-type calcium currents and is expressed in the form of rebound low-threshold spikes (LTSs). LTSs can be elicited *in vitro* in LGN thalamocortical cells by injecting hyperpolarizing current pulses into the cell, resulting in the deinactivation of the T-type current followed by activation that occurs during repolarization of the membrane (Jahnsen and Llinás, 1984a,b; Kim et al., 1995; Bal et al., 1995a,b). Figure 1A shows voltage responses elicited from LGN neurons by a series of hyperpolarizing current prepulses of one second duration followed by a passive repolarization of the membrane upon termination of the hyperpolarizing current. Depending on the voltage range of the hyperpolarization, rebound LTSs are elicited (Fig. 1A). In the presence of ethanol (20 mM) the stereotypical LTS is completely eliminated without changing the membrane input resistance (Fig. 1B). This effect was consistently seen in several cells sampled with current clamp recordings from the ferret LGN ($n = 3$). Although this result was highly suggestive of ethanol's ability

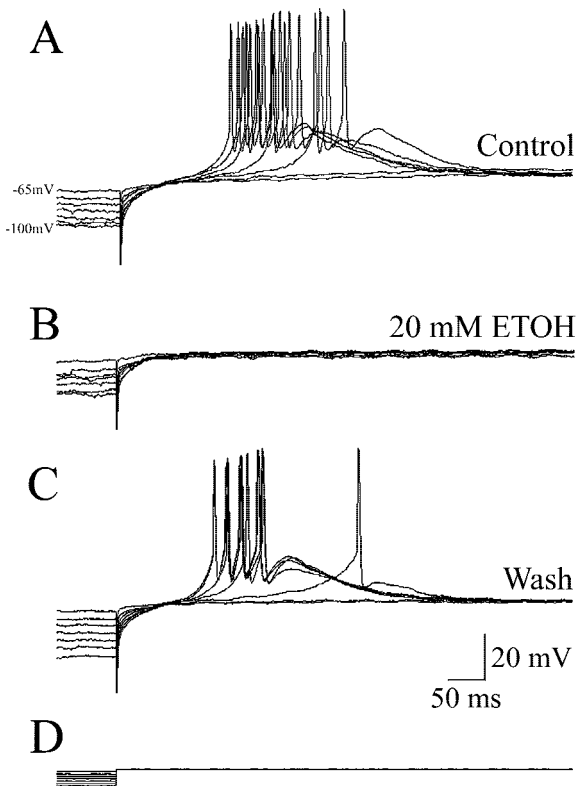


Fig. 1. Ethanol reduces LTSs generated by rebound T current. Voltage traces recorded in current clamp mode illustrate LTSs elicited by depolarization back to the holding potential ($-61 \text{ mV} \pm 2 \text{ mV}$) after a series of hyperpolarizing current steps (0.1 nA steps, 1 s , over a range of -100 mV to -65 mV). A, control traces show robust LTS activity that is blocked upon exposure to 20 mM EtOH (B); C, LTSs recovered within 10 min ; D, current step protocol used to generate rebound LTSs.

to disrupt burst firing through the T-type calcium channel, we used voltage-clamp recording techniques to confirm and characterize ethanol interactions with the native T-type calcium current.

Characterization of the T-Type Current. The voltage-dependent properties of the native T-type current recorded from LGN thalamocortical cells are summarized in Fig. 2. Representative traces recorded from T-type current activation (Fig. 2A) and inactivation (Fig. 2C) in a single cell are shown with corresponding voltage step commands shown in Fig. 2B and Fig. 2D, respectively. Average peak inactivation ($n = 15$, squares) and activation ($n = 25$, triangles) currents were normalized to the peak of the maximally available current (I/I_{max}). This relative current was plotted as a function of prepulse potential and fitted with a Boltzmann distribution as shown in Fig. 2E. Our results indicate a T-type current activation slope coefficient (k) of 2.5 and that the T-type current was half-maximally activated ($V_{1/2}$) at -65 mV . Furthermore, the T-type current inactivation slope coefficient (k) was 3.8 , and the T-type current inactivation had a $V_{1/2}$ of -80 mV .

Our results for baseline T-type current activation and inactivation kinetics correspond well with previous observations of native T-type channels examined *in vitro* (Coulter et al., 1989; Hernández-Cruz and Papé, 1989). Because we recorded at a temperature and with calcium concentrations that more closely approximate physiological conditions, the voltage dependence of T-type channel activation and inacti-

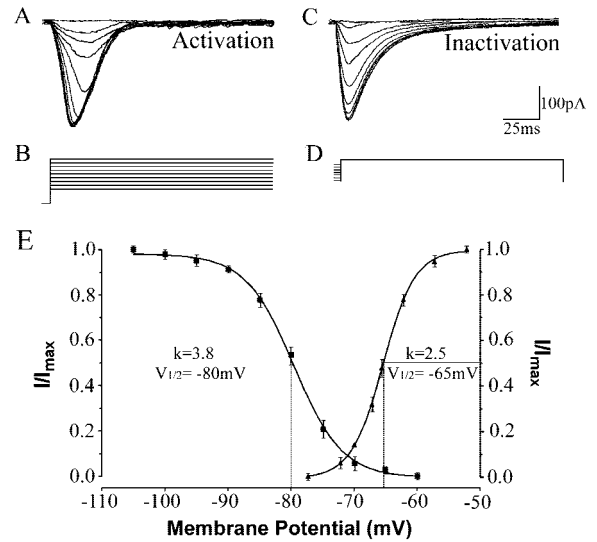


Fig. 2. Voltage dependence of activation and steady-state inactivation of T-type current in LGN neurons. Activation kinetics of the T-type current were recorded by responses to a series of 5 mV increments in command potential from -85 mV to -55 mV initiated from a prepulse membrane potential of -95 mV . Inactivation kinetics were characterized from responses to depolarization to -60 mV from a series of membrane potentials beginning at -130 mV and incrementing in 5 mV steps. Under both conditions, the prepulse interval was 1 s . Representative traces showing activation (A) and inactivation (C) of the T-type current using the command potential (mV) protocols are indicated in panels B and D, respectively. E, currents obtained at each holding potential were normalized to the peak current (I/I_{max}) and plotted using Boltzmann functions for T current activation (right curve, $n = 25$) and inactivation (left curve, $n = 15$) to obtain the slope (k) and $V_{1/2}$ (values of each are indicated on the graph). Overlap of the activation and inactivation curves revealed a window current that could allow T-type current generation near resting membrane potentials. Error bars represent the standard error of the mean.

vation are shifted to a more hyperpolarized range. The slopes of the corresponding Boltzmanns were also steeper than those typically reported at room temperature and lower calcium concentrations (Crunelli et al., 1989). This variation is particularly apparent when examining native or expressed T-type channels in isolated cell preparations (see Perez-Reyes (2002) for review).

In addition to the activation and inactivation voltage protocols used to isolate the T-type current, we used the calcium channel blocker mibefradil ($10 \mu\text{M}$; $n = 4$) and Ni^{2+} ($200 \mu\text{M}$; $n = 6$) in a subset of cells to further verify we were isolating the T-type current for analysis (data not shown). Bath application of mibefradil and Ni^{2+} completely attenuated the calcium current obtained within the above voltage-clamp parameters, and the LTS recorded in current clamp, thus indicating that they are mediated by the T-type Ca^{2+} channel (see Hernández-Cruz and Papé, 1989), this attenuation recovered. These results highlight the voltage-dependent nature of both activation and inactivation of the T-type current and are fundamentally consistent with descriptions of the T-type current elicited using similar voltage protocols in thalamic slices prepared under similar conditions (Coulter et al., 1989; Hernández-Cruz and Papé, 1989). The overlap that we observed between the activation and inactivation curves (Fig. 2E) indicates the presence of a “window” current that has been reported by others (Coulter et al., 1989; Hughes et al., 1999). The presence of this window current implies that active T-type channels may be available at resting membrane

potentials, and under certain circumstances, this could impart a form of membrane bistability as well as a potential mechanism for amplification of synaptic inputs.

Ethanol Differentially Affects the T-Type Current.

To control for the possible effects of ethanol on other voltage-dependent properties of thalamic neurons, we recorded ethanol effects in LGN cells in voltage-clamp mode using whole cell patch methods, which allowed us to isolate the T-type current, as described above. Ethanol both enhanced and attenuated the T-type current depending on the concentration delivered. We found that lower concentrations of ethanol (2.5, 5, and 10 mM) enhanced the peak amplitudes of the T-type current whereas higher ethanol doses (20 and 50 mM) decreased peak current amplitudes. The differential effect of ethanol on the T-type current across the entire range of doses used in this study is graphically summarized in Fig. 3. Relative increases or decreases in peak current amplitude elicited during T-type current activation were normalized to control activation values and then averaged to obtain a relative change in T-type current amplitude for all cells tested at each ethanol concentration. Using paired *t* tests ($p < 0.01$) to evaluate statistical significance, the mean peak T-type current amplitudes were significantly increased (41% and 40%) with 5 and 10 mM doses, respectively, compared with control values. In contrast, mean peak T-type current amplitudes decreased significantly (−28% and −52%) with 20 and 50 mM doses, respectively.

Further examination of the 5 and 50 mM ethanol exposure conditions highlights the highly contrasting effects of ethanol concentration on T-channel activation. We examined the relative time course of ethanol's influence on T-type currents by plotting the change in peak current activation normalized to peak control activation measured every minute for populations of cells exposed to each of these ethanol concentrations (Fig. 4, A and B). The ethanol concentration and the duration of exposure are indicated above the x-axis in each graph, and the error bars indicate standard error of the mean. Representative current traces located above the mean normalized

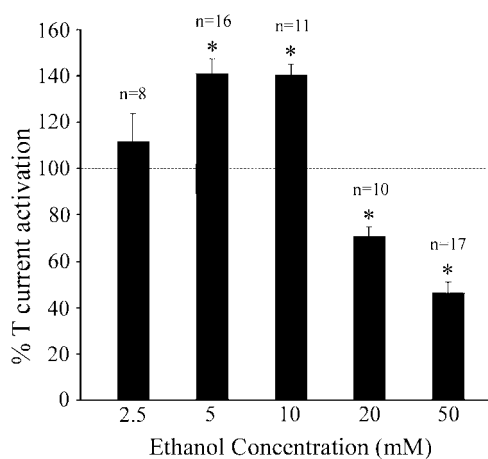


Fig. 3. Ethanol has a concentration-dependent effect on the T current. Histogram showing the mean peak amplitude elicited from population T current activation recorded in the presence of a range of EtOH doses. Peak T current amplitude is plotted as a percent change relative to control T current values. 2.5, 5, and 10 mM EtOH concentrations increased T current amplitude, whereas 20 and 50 mM concentrations decreased the T current. Error bars represent the standard error of the mean. Asterisks denote values of EtOH concentrations that resulted in a significant change from control values.

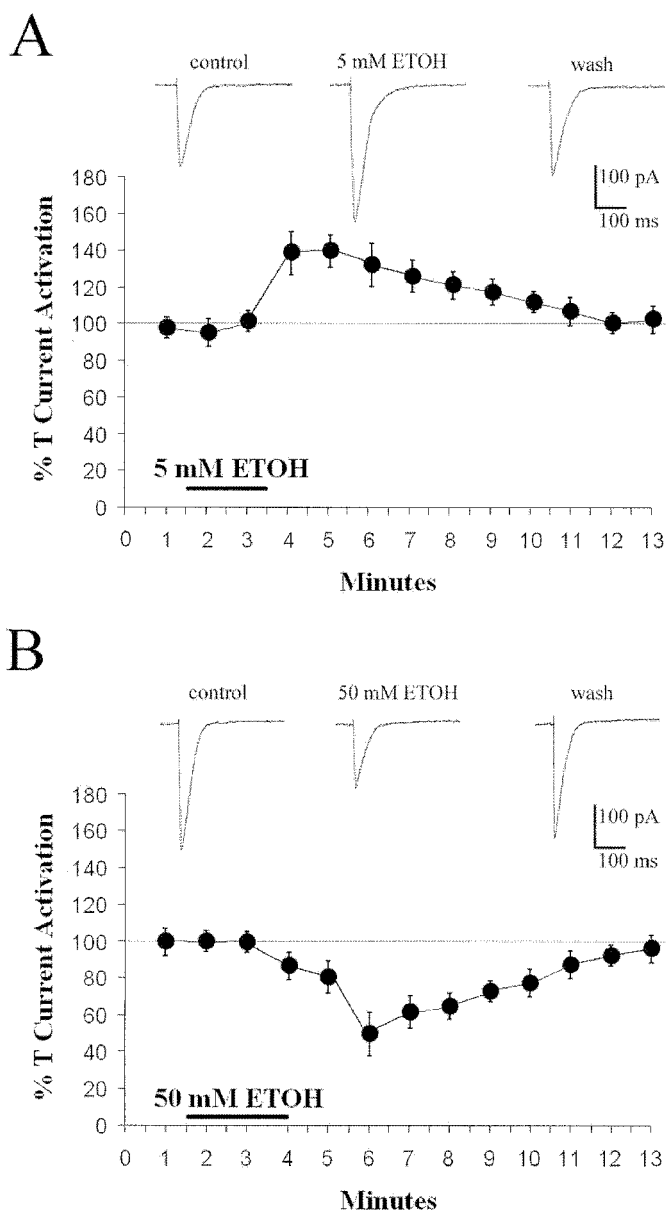


Fig. 4. Timecourse of the concentration-dependent effect of EtOH on the T current. The amplitude of peak T current activation is plotted as a percent change relative to control T current values and is shown as a function of time before, during, and after EtOH delivery. A, representative current traces (above graph) before, during, and after exposure to 5 mM EtOH show a significant increase in peak T current amplitude with recovery on washout. Pooled data from 16 cells shows a 41% increase in mean T current amplitude, which recovered on average 7 min after drug application. B, representative current traces (above graph) before, during, and after exposure to 50 mM EtOH show a significant decrease in peak T current amplitude with recovery on washout. Pooled data from 17 cells shows a 52% decrease in mean T current amplitude, which recovered on average 9 min after drug application. Error bars represent the standard error of the mean.

activation values illustrate the relative enhancement (Fig. 4A) or attenuation (Fig. 4B) of peak T-channel activation for a given cell. The onset of both the enhancement and the attenuation of T-type currents by their respective ethanol doses coincided very closely with the calculated rate of solution exchange in the recording chamber, thereby indicating a rapid action of ethanol on T-channels. Surprisingly, we did not observe a brief increase in peak current during the first 1

to 3 min after initial application of the 50 mM concentration of ethanol, when the concentration of ethanol in the slice would be passing through lower concentrations. This may be due to the design of our drug application, the small size of our recording chamber, and the fact that we sampled at 1-min intervals. With total bath substitution, we were very rapidly replacing the “control ACSF” with the “ethanol ACSF” at the concentration being tested, which may have obscured any transient increases. A more rigorous examination using increased temporal resolution and a more gradual and localized ethanol delivery system would likely reveal the transient increase followed by decrease in T-type current activation upon the addition of higher concentrations of ethanol.

To assess whether the enhancement or attenuation of T-type current by 5 and 50 mM ethanol concentrations, respectively, was due to a shift in the voltage dependence of steady-state activation and inactivation, we fit the current activation and inactivation data with Boltzmann functions (Fig. 5). In the presence of 5 mM ethanol, the activation threshold (approximately -72 mV), half-maximal current activation ($V_{1/2} = -63.5$ mV), and slope coefficient ($k = 2.3$) were not significantly different (paired t test, $p > 0.01$) from control activation values (Fig. 5A). In contrast to the lack of effect on T current activation by 5 mM ethanol exposure, the voltage dependence of the inactivation curve was significantly altered. The voltage at which half of the channels were inactivated ($V_{1/2} = -75$ mV) was shifted significantly in the depolarized direction by 5 ± 0.8 mV (paired t test, $p < 0.01$); however, the slope coefficient ($k = 3.7$) was not significantly different (paired t test, $p > 0.01$) from the control inactivation values (Fig. 5A). The voltage shift in T-type current inactivation in the presence of 5 mM ethanol may increase the number of channels available to be open during depolarization providing a mechanism for the increased T current measured during this low-dose exposure.

We also examined whether any changes in the voltage dependence of steady-state activation and inactivation accounted for the attenuation of T-type current in the presence of 50 mM ethanol. Using the same electrophysiological protocols and Boltzmann fitting procedure described above for the 5 mM exposure, we fit current activation and inactivation data in the presence of 50 mM ethanol (Fig. 5B). In contrast to the 5 mM results, we observed no significant change (paired t test, $p > 0.01$) in the voltage dependence of the activation or inactivation curves, the slope coefficients ($k = 2.5$, activation; $k = 3.8$, inactivation) or the voltage at which half of the channels were activated ($V_{1/2} = -66$ mV) or inactivated ($V_{1/2} = -80$ mV) from control activation and inactivation values. Therefore, in contrast to the 5 mM enhancement of the T-type current, the attenuation seen with 50 mM ethanol exposure does not appear to result from a shift in the voltage dependence of steady-state activation or inactivation.

Within-Cell Findings. The lateral geniculate nucleus is known to contain local interneurons as well as two morphologically and physiologically distinct thalamocortical neurons (for review, see Sherman and Koch, 1998), which may have accounted for the differential T-type current response to ethanol we observed. To address this possibility, in a subset of cells we exposed single neurons to multiple doses of ethanol. Within-cell observations showed both attenuation and en-

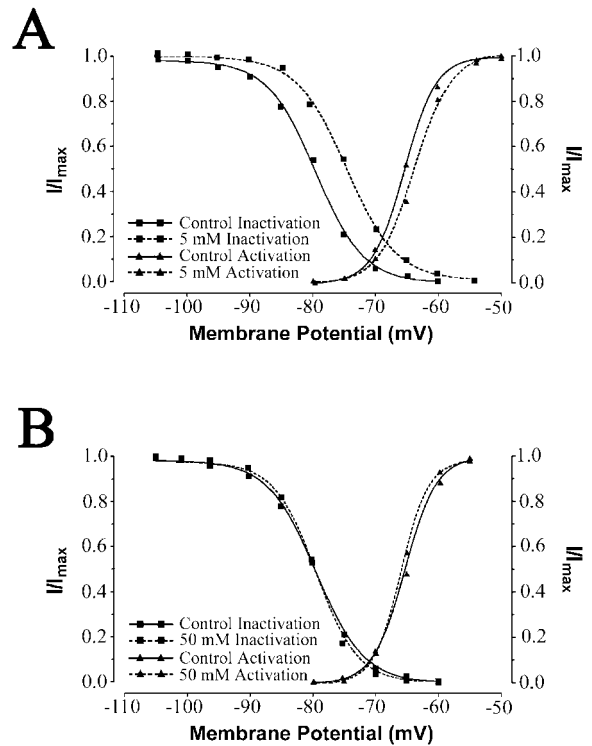


Fig. 5. The low dose of ethanol shifts the voltage dependence of steady-state inactivation of the T-type calcium current. Voltage protocols designed to elicit steady-state activation and inactivation (see *Materials and Methods* for full description) were performed before and after 5 mM (A) and 50 mM (B) EtOH exposure. Control (solid lines) and EtOH exposed (dashed line) currents obtained at each holding potential were normalized to the peak current (I/I_{\max}) and plotted using the best-fit Boltzmann function for T-current activation (\blacktriangle) and inactivation (\blacksquare) to obtain the slope (k) and $V_{1/2}$ values. A, in the presence of 5 mM ethanol ($n = 16$), the voltage at which half of the channels were inactivated ($V_{1/2} = -75$ mV) was shifted significantly in the depolarized direction by $5 \text{ mV} \pm 0.8 \text{ mV}$ (paired t test, $p < 0.01$); however, the slope coefficient ($k = 3.7$) was not significantly different (paired t test, $p > 0.01$) from the control inactivation values. The activation threshold (approximately -72 mV), half-maximal current activation ($V_{1/2} = -63.5$ mV), and slope coefficient ($k = 2.3$) were not significantly different (paired t test, $p > 0.01$) from control activation values. B, in the presence of 50 mM ethanol ($n = 17$), there was no significant change (paired t test, $p > 0.01$) in the voltage dependence of the activation or inactivation curves, the slope coefficients ($k = 2.5$ activation; $k = 3.8$, inactivation), or the voltage at which half of the channels were activated ($V_{1/2} = -66$ mV) or inactivated ($V_{1/2} = -80$ mV) from control activation and inactivation values.

hancement irrespective of the order of administration of the high and low concentrations. In the first experiment, the peak T-type current activation was measured before ethanol application, during 20 mM exposure, during an interim wash period, during 5 mM exposure, and during a second wash period (Fig. 6A, $n = 3$). Under this experimental paradigm, the 20 mM dose reduced the mean peak activation by $39 \pm 16\%$ from baseline, followed by a return to baseline activation values, and a subsequent $34 \pm 14\%$ increase in mean peak activation from the initial baseline values. The increase in T-type calcium current amplitude recovered to baseline activation values. In the second experiment, the same design described above was used, with 5 mM ethanol exposure followed by a 50 mM ethanol exposure; this exposure paradigm yielded $42 \pm 10\%$ increase followed by a $32 \pm 10\%$ decrease in mean peak T-type current activation from the initial baseline values, respectively (Fig. 6B, $n = 4$). Thus, these experiments demonstrated that the differential effect of ethanol on

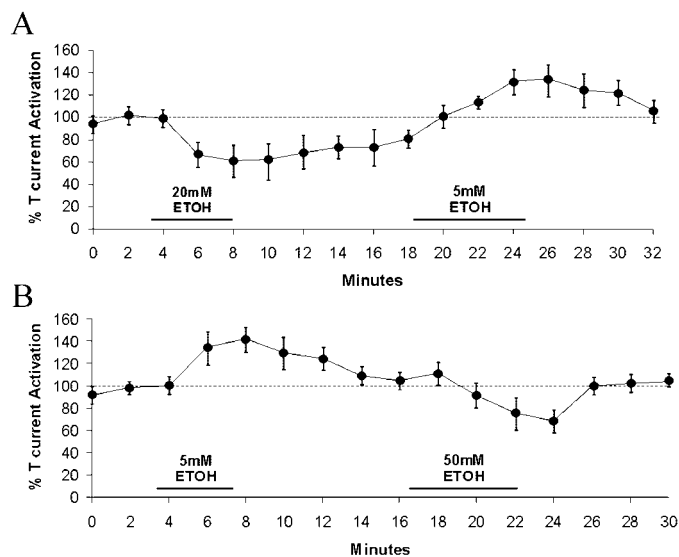


Fig. 6. The concentration-dependent effect of EtOH within the same cell. The peak amplitude elicited from T current activation was measured from cells receiving multiple doses of EtOH. The resulting amplitudes were normalized to control T current amplitudes and plotted as a function of time. A, after baseline T current activation values were obtained, cells were exposed first to 20 mM, allowed to recover, and then exposed to 5 mM EtOH. Data pooled from 3 cells show a 39% reduction followed by 34% enhancement of T current amplitude, which recovered to baseline activation values. B, peak T current activation amplitude data pooled from 4 other cells that received first a 5 mM then a 50 mM EtOH exposure show a 42% increase followed by a 32% decrease, respectively, in T-type current activation from baseline values. Error bars represent the standard error of the mean.

the T-type current occurs within the same cell, and the relative enhancement or attenuation of the current occurs regardless of the order of ethanol treatment.

Discussion

Our study examined the effects of ethanol on native T-type calcium currents in the dorsal lateral geniculate nucleus. Although we have not characterized the specific T-channel subunits involved in our observed responses, hybridization studies suggest that thalamic relay neurons primarily express $\alpha 1G$ mRNAs, whereas other transcripts are expressed in the thalamic reticular nucleus (Talley et al., 1999). We observed unique dose-dependent effects on the native T currents in the LGN. At low clinically relevant concentrations, ethanol enhanced T-type currents by 24% at 5 mM and 23% at 10 mM. The enhancement of T-type channels was due in part to a depolarizing shift in $V_{1/2}$ of the inactivation curve. This shift in the inactivation curve suggests that at low concentrations ethanol enhances T-type currents, in part by increasing channel availability. We did not observe significant changes in the activation curves of the native T-type current. No significant changes in k by ethanol were observed, indicating that the voltage sensor of the T-type channels was not affected.

T-type Ca^{2+} channels exhibit a “window current” at voltages where channels are not completely inactivated. In these ranges, which are typically near resting membrane potential (denoted in Fig. 5 by the region of overlap between the steady-state inactivation and activation curves), T-channels are available to open. Although the extent of window current is dependent upon experimental conditions (Williams et al.,

1997), we have attempted to define our experimental conditions to be as close as possible to the temperature and ion distribution found in normal neuronal environments. Our measurements of window current from native T-type channels are consistent with similar experiments performed in other thalamic preparations (Crunelli et al., 1989; Tarasenko et al., 1997; Perez-Reyes, 2002). One consequence of the depolarizing shift in the steady-state inactivation with low ethanol concentrations is that the window current is significantly enhanced with presumably more available T current at resting membrane potentials. Increased T current availability with low ethanol concentration exposure could lead to increased spindle wave activity and duration of Stage II sleep. This is precisely what has been reported in the clinical literature with moderate doses of ethanol (0.55 g/kg of body weight) administered to nonalcoholic test subjects resulting in a decrease in rapid eye movement (REM) sleep and an enhancement of slow wave sleep time and spindle wave activity (Stone, 1980; Papineau et al., 1988; Landolt et al., 1996).

In contrast to the effects of ethanol at low concentrations described above and in agreement with prior observations examining the effect of acute and chronic ethanol exposure on T-type calcium channels (Twombly et al., 1990; Littleton et al., 1992; Knott et al., 2002), at high concentrations, we observed a significant dose-dependent reduction in the T-type current (Fig. 3). Furthermore, this reduction occurred without significant changes in activation or inactivation curves (Fig. 5B), similar to the kind of dose-dependent block observed with volatile anesthetics (McDowell et al., 1999). Thus ethanol influences on T-type channels appear to take a dual course through two different mechanisms, one of which operates at low concentrations and involves changes in the inactivation kinetics of the native T-type channels; the other of these mechanisms may engage when ethanol concentration exceeds ~ 10 mM and produces a block of whole cell currents without significantly altering channel kinetics. A more detailed assessment of these dual mechanisms at the macroscopic and single channel level along with a more complete description of dose-response relationships will help to determine more precisely how ethanol interacts with the T-type channel. The determination of the behavior of the channel at the transition point between enhancement and inhibition will be of particular interest; this should occur somewhere between 10 and 20 mM (Fig. 3).

T-type calcium currents underlie the phenomenon of thalamic burst firing. This burst firing factors prominently in normal thalamic sleep rhythms (Steriade and Llinás, 1988; Steriade et al., 1985; Bal et al., 1995a,b) as well as pathological rhythms (Jeanmonod et al., 1996). In humans, spindle oscillations in the thalamus that populate Stage II sleep are composed of such high-frequency bursts of action potentials that occur at ~ 7 to 14 Hz, with an interspindle frequency of ~ 0.1 to 0.4 Hz. Intracellular recordings from both thalamic reticular neurons and thalamic relay neurons have revealed that cells within both nuclei contribute to the generation of these waves through cyclical activation of burst discharges that are produced by a T current that is common to cells of both nuclei (Jahnsen and Llinás 1984a,b; Bal et al., 1995a,b; Kim et al., 1995). This network activity is transmitted to layer 4 of cortex by the “output” of the circuit, the thalamo-cortical axons of relay neurons, where surface EEG record-

ings can detect the characteristic oscillation. It is clear that the T-type currents are important in governing the sub-threshold neuronal excitability, and in regulating the frequency and pattern of spike discharges (Jahnsen and Llinás, 1984a,b; Hughes et al., 1999). Therefore, even modest enhancement of T-type currents would be expected to significantly enhance such firing, since these currents elicit high-frequency sodium spikes that fire in an all-or-none manner. Conversely, reduction of these currents may relate to reductions in spindling seen clinically in the latter portion of the sleep period (Stone, 1980; Williams and Rundell, 1981; Vitello, 1997).

Virtually every type of sleep disruption occurs in alcohol-dependent patients (Allen et al., 1971; Gillin et al., 1990; Landholt et al., 1996). Typically, these individuals take a longer time to fall asleep and show decreased sleep efficiency, shorter sleep duration, and reduced amounts of slow wave sleep when compared with healthy controls. Their sleep patterns are fragmented, and the typical time course of EEG delta wave activity is severely disrupted. The amount of REM sleep may be reduced or increased. Sleep changes can persist during months or years of abstinence, and recent studies indicate that certain alterations in sleep architecture, as well as subjective sleep complaints, predict relapse to alcoholism (Roehrs et al., 1999). Therefore, understanding the fate of slow wave sleep and particularly associated spindle wave oscillations may yield important insight into the neuroadaptational changes that may occur with chronic alcohol consumption. Sleep disturbances associated with withdrawal are characterized by lower slow wave sleep and higher levels of REM sleep (Allen et al., 1971). Higher levels of REM predict relapse in patients, in part because this form of sleep appears to have less "quality" than slow wave sleep (Akerstedt et al., 1997). The absence of particular forms of sleep may thus partially drive relapse. Stage II sleep, which is the most populated with spindles, also has the most intimate relationship with REM in that this form alternates with REM during the latter part of the night (Hobson, 1999). Although it is premature to equate the influence of ethanol on T-type current with the ultimate fate of slow wave sleep, the central role of T-type current in spindle oscillations argues that even subtle enhancement or diminution may presage changes in the quantity of spindle-like sleep in drinkers.

The differential effect of ethanol concentration we observed might also suggest an important difference in the way the sleep circuitry of the brain responds to ethanol in drinkers that consume low versus high amounts of ethanol. Low concentrations of ethanol enhance T-type current, which we would predict would enhance spindle rhythms, and may contribute to the sedative effect of ethanol. The reduction of T-type current we see at higher ethanol concentrations, if prolonged, might underlie long-term neuroadaptational changes in the circuitry that disrupt normal sleep patterns that depend upon T-type channels.

The acute effects of ethanol on T-type current function presented here have significant implications regarding alterations in sleep rhythms reported in the clinical literature. It is important to note that normal sleep architecture is a complex phenomenon regulated by many brain regions involving intricate synaptic circuits whose function depends upon multiple neurotransmitter systems, including GABA,

serotonin (5-hydroxytryptamine), adenosine, glutamate, and nitric oxide. Without diminishing the role of these other neurotransmitter effects, our current findings suggest that voltage-gated channels may play an equally important role on their own or in conjunction with these neurotransmitter systems, providing a potential therapeutic target to address sleep disturbances resulting from alcohol abuse. Further studies are needed to clarify the relationship between sleep, sleep abnormalities and alcoholism, to establish new approaches to improve sleep in alcohol-dependent patients, and to prevent withdrawal reactions that affect sleep during abstinence.

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